PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/003684	06.04.2004	11.04.2003

International Patent Classification (IPC) or both national classification and IPC
A61K49/08

Applicant
BRACCO IMAGING S.P.A.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,8
	No:	Claims	1-3,5,6,7,9
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V.

The following document have been cited in the search report. Where reference is made to them, the following numbering is used; reference is made to the relevant passages emphasized in the Search Report:

D1: WO 96/38184 A (FRASER SCOTT ;JACOBS RUSSELL (US); MEADE THOMAS (US); CALIFORNIA I) 5 December 1996
D2: WO 03/013617 A (SCHERING AG) 20 February 2003
D3: EP-A-1 106 186 (MALLINCKRODT INC) 13 June 2001
D4: WO 02/40060 A (BRACCO INT BV) 23 May 2002
D5: US-A-5 271 924 (IWAI KUMIKO ET AL) 21 December 1993
D6: WO 02/43775 A (UNIV TEXAS) 6 June 2002
D7: EP-A-1 331 012 (BRACCO IMAGING S P A) 30 July 2003
D8: ZHANG S ET AL: "A NOVEL PH-SENSITIVE MRI CONTRAST AGENT" ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, VERLAG CHEMIE. WEINHEIM, DE, vol. 38, no. 21, 2 November 1999 (1999-11-02), pages 3192-3194, XP000864995
D9: WO 99/55230 A (NAGY JAMES I) 4 November 1999
D10: AIME S ET AL: "PARAMAGNETIC LANTHANIDE(III) COMPLEXES AS PH-SENSITIVE CHEMICAL EXCHANGE SATURATION TRANSFER (CEST) CONTRAST AGENTS FOR MRI APPLICATIONS" MAGNETIC RESONANCE IN MEDICINE, ACADEMIC PRESS, DULUTH, MN, US, vol. 47, no. 4, 2002, pages 639-648, XP001151672
D11: WO 00/66180 A (ALETRAS ANTHONY H et al.) 9 November 2000 (2000-11-09)
D12: WO 92/13572 A (DIATECH INC) 20 August 1992 (1992-08-20)
D13: AIME S ET AL: "Non-covalent conjugates between cationic polyamino acids and GdIII chelates: a route for seeking accumulation of MRI-contrast agents at tumor targeting sites." CHEMISTRY 14 JUL 2000, vol. 6, no. 14, 14 July 2000, pages 2609-2617, XP002287529
D14: ALLEN MATTHEW J ET AL: "Synthesis and visualization of a membrane-permeable MRI contrast agent." JOURNAL OF BIOLOGICAL INORGANIC CHEMISTRY : JBIC. SEP 2003, vol. 8, no. 7, September 2003, pages 746-750, XP002287530
D15: ALLEN MATTHEW J ET AL: "Cellular delivery of MRI contrast agents." CHEMISTRY & BIOLOGY. MAR 2004, vol. 11, no. 3, March 2004 (2004-03), pages 301-307, XP002287531

Novelty (Art.33(2)) PCT

D1 discloses (see claims 1, 2, 4, 10, 13) complexes of DOTA with the same paramagnetic metals of the present application, covalently conjugated to sugars (figure 3) and polyaminoacids (figures 5, 6). Delivery in liposomes is also disclosed (see page 45, line 2). DOTEP, a DOTA-phosphate, is also mentioned (see page 21, structure 5). Since the sugars and the polyaminoacids used in D1 have "mobile protons in exchange with water", the compounds disclosed in D1 fall in the definition of claim 1. In view of

this prior art, the subject matter of claims 1,2,5,7,9 is not new.

D2 discloses, (see page 13, lines 20-26; page 17, compounds II and III; table 1, claims 1,11,-13, table 1) conjugates of paramagnetic metal chelates and a "biomolecule", (a molecule having biological activity). Since the "biomolecules" shown in table 1 have mobile protons in exchange with water, (see for example the peptide N.68 comprising arginine), the compounds disclosed in D2 fall in the definition of claim 1. In view of this prior art, the subject matter of claims 1,2,7,9 is not new.

D3 (see paragraphs 9, 12, 17,20, examples 1,7, claims 1-3,8,13,14) discloses non - covalent bio-conjugates of a lanthanum or of an iron paramagnetic chelate with a carrier; one of the preferred carriers is polyarginine. Such conjugates are used as NMR imaging agents. In view of this prior art, the subject matter of claims 1-3, 6, 9 is not new.

D4 (see page 2, lines 4-11; page 5, lines 1-4; page 7, lines 17-25; claims 1,2,5) discloses contrast agents (also for MRI) comprising a DOTA paramagnetic chelate conjugated to an ascorbic acid residue (comprising mobile protons in exchange with water). In view of this prior art, the subject matter of claims 1, 7, 9 is not new.

D5 discloses conjugates of a chitosan oligosaccharide, or of a galactosamino - oligosaccharide, conjugated to DOTA complexes of the relevant metals and the use of these conjugates as imaging agents. The chitosan falls in the definition "polyamine" and in the definition "polysaccharide" of claim 2. In view of this prior art, the subject matter of claims 1, 2, 7, 9 is not new.

D6 discloses contrast agents comprising complexes of paramagnetic metals and DOTA comprising appended amido moieties exchanging protons with water. In view of the very general definition of the term "substrate" in claim 1, the compounds of D6 also fall in the definition of claim 1. In view of this prior art, the subject matter of claims 1, 2, 7, 9 is not new.

D13 discloses (see abstract, scheme 1, results) contrast agents made by a non-covalent conjugate between a cationic polyaminoacid (polyarginine is a preferred one) and a gadolinium chelate. This agent falls into the definition of claims 1,2,3,6,9 and is

novelty destroying for these claims.

Inventive step (Art.33(3) PCT)

The problem underlying the present application is the provision of new contrast agents suitable to be used in saturation transfer techniques for NMR imaging. As a solution, the inventors propose contrast agents comprising a moiety bearing at least one mobile proton in exchange with water.
As mentioned above, contrast agents of these type are already known from the prior art, and most of the claims of the present application are not new.
Furthermore, it appears that the subject matter of the present claims which is still new (for example the one relating to an agent as the one shown in formula 8) is not characterized by any new additional technical feature providing any new technical effect over the prior art. The use of poly-arginine sequences in paramagnetic conjugates is also well known (see for example D3, D13, and D12). In the absence of any new technical effect, the subject matter of the claims which is still new is considered an obvious alternative to the prior art, and may not be considered to involve an inventive step.

Industrial Application

The subject matter of the application is industrially applicable.